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# Recovery of Viruses from the Missouri River

Environmental Protection Agency, Cincinnati, OH Water Qual Off.

Feb 71

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RECOVERY OF VIRUSES FROM THE MISSOURI RIVER

ENVIRONMENTAL PROTECTION AGENCY  
WATER QUALITY OFFICE  
CINCINNATI, OHIO

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UNITED STATES GOVERNMENT

# Memorandum

ENVIRONMENTAL PROTECTION AGENCY, Water Quality Office  
4676 Columbia Parkway, Cincinnati, Ohio 45226

*Dugan*  
1-EPA-WQ-  
SEC-CY-71

TO : J. M. Rademacher  
THRU : E. N. Kari  
F. M. Middleton  
FROM : G. Berg  
Chief of Virology, AWTRL

DATE: February 25, 1971

SUBJECT: \* Recovery of Viruses from the Missouri River

Enclosed is a report on our cooperative endeavor to assess the virus hazard along the Missouri River. This study was the first of its kind and, in my judgment, constitutes a hallmark effort.

It could not have been done without the extraordinary work of Carl Walter and others of your Region, some of whom exposed themselves to serious dangers in obtaining adequate samples from sometimes swift and icy waters.

I am pleased that the results of this mutual undertaking served your enforcement efforts so effectively.

It is greatly significant that we were able to demonstrate a virus hazard with techniques capable of detecting only a small fraction of the viruses that are present. It is clear, if regrettable, that unless more funds become available for technic development, we are going to be unable to detect even relatively large numbers of viruses flowing into our water intakes along all of the waterways in this country.

To us, this effort was especially valuable, for it allowed us to crystallize approaches and needs for the even more productive efforts now under way on the Mississippi River. Moreover, it made possible important technic developments that we might have never realized were necessary had we, as a research laboratory, not been directly involved in this field effort. It is clearly imperative for our laboratories to be directly involved in field operations if we are to perform with a maximum effectiveness in resolving the problems that face our personnel in the field.

We look forward to serving your Region whenever again we can.

*Gerald Berg*  
Gerald Berg, Ph.D.

Enclosure

cc: Comm., WQ, EPA, Attn: Ass't. Comm.  
for Enf. & Stds. Comp.  
Dir., NFIC, WQ, EPA, Cincinnati, O.  
Dir., DFI-DC, EPA, WQ, Denver, Colo.  
(Fwd. by WQ, EPA, Region VII, Kansas City " "  
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## THE VIRUS HAZARD ON THE MISSOURI RIVER

Gerald Berg, Daniel R. Dahling, Donald Berman, and Carl Walter

A single infectious virus excreted by a human is capable of infecting other humans who consume that virus (Table 1)(1). The presence of a single such virus in water that people consume, therefore, constitutes a clearcut hazard to health and well-being.

Fecal coliforms always occur in the gastrointestinal tracts of warm blooded animals. Certain viruses and pathogenic bacteria may or may not be present at the same time. Fecal coliforms serve as indicators that viruses or pathogenic bacteria may be present. Thus, evidence of fecal coliforms in water is only an indication that hazardous agents may be present. Evidence constituted by many other biological and chemical indicators of pollution is often equivocal too, because in themselves, these indicators usually cannot be shown to be injurious to health. When no one line of evidence is conclusive, multiple lines of such evidence must be relied upon to support enforcement actions on the premise that the sum of the many such lines of evidence will stand stronger than any one line could stand alone.

Viruses are a different matter. Each virus is capable of producing infection. Each virus is thereby a dangerous pollutant. Thus, the detection of a single virus particle is the detection of a dangerous pollutant.

The detection of a virus in a sewage effluent ejected into a waterway constitutes a clear and present danger to health in the area of the outfall, immediately downstream of the outfall, and in communities → 02

well downstream when that virus will survive in the stream long enough to reach the downstream communities. Even when downstream transmission is not in evidence, the presence of viruses in an effluent and in a receiving water a short distance downstream of the outfall from which the effluent is discharged is a hazard, constitutes an adulteration of stream quality in that area, and thereby in itself demands remedial action.

*> The report discusses materials, methods, identification, and recovery. <—*

#### MATERIALS AND METHODS

Collection of samples. All samples were collected by Region personnel. Raw influent and effluent samples were collected in cubitainers from primary treatment plants along the Missouri River and couriered to Cincinnati by air or transported by truck when river water samples were also taken. Samples were kept cold during transport.

Most sewage and effluent samples were processed immediately upon reaching the laboratory, but some were stored at -70 C before processing.

Large samples of water were collected in 55-gallon plastic-lined drums from selected locations on the Missouri River and trucked overnight to Cincinnati. In some instances, field filtrations were achieved with the equipment described below and the filter sandwiches were returned to Cincinnati, along with the silt collected on the pre-filters, for processing.

#### Recovery of viruses from sewage and treatment plant effluents.

Each two-liter sample of sewage or treatment plant effluent was filtered through a Millipore AP 20 fiberglass prefilter and an MF 0.45  $\mu$  membrane

filter. Prefilters and filters were pretreated with 0.1% Tween-80 to prevent virus adsorption to the filters and then rinsed with distilled water prior to contact with the samples. Ten ml of an  $\text{Al}(\text{OH})_3$  gel, prepared in McIlvaine's buffer\* by procedures described elsewhere (2), was added to each filtered sample and each suspension was stirred with a magnetic stirrer for an hour. The  $\text{Al}(\text{OH})_3$  precipitates, to which virus had adsorbed, were collected by filtration on MF 0.45  $\mu$  membranes and removed with a spatula. The membranes were washed with 10 ml of a cell growth medium, and the washings were added to the corresponding  $\text{Al}(\text{OH})_3$  precipitates. The suspensions of medium and  $\text{Al}(\text{OH})_3$  were diluted 1:5 and inoculated onto cell cultures, 1 ml per culture, for assay by the plaque technic.

Five grams of fraction 5 bovine albumin and 100 ml of 1% aqueous protamine sulfate were added to each sample from which the  $\text{Al}(\text{OH})_3$  had been filtered and the suspensions were stirred for 30 minutes with a magnetic stirrer. The precipitates that formed were then collected on Tween-80-treated Millipore AP 20 fiberglass prefilters, and 1 ml of 1 M NaCl was filtered through each pad to dissolve the precipitates and elute the viruses. Each pad was subsequently washed with 6 ml of distilled water which were added to the corresponding dissolved precipitate, and the total volumes were inoculated onto cell cultures, 1 ml per culture, for assay by the plaque technic (3).

In some tests, the  $\text{Al}(\text{OH})_3$  and protamine sulfate procedures were applied to separate samples of effluent, because at the time the

\* McIlvaine's buffer consisted of 0.05 M  $\text{Na}_2\text{HPO}_4$  and sufficient citric acid to bring the pH to 6.

studies were done, it was believed that the  $\text{Al}(\text{OH})_3$  procedure would recover only small viruses and the protamine sulfate procedure would recover only large viruses. Subsequently, it was shown that the  $\text{Al}(\text{OH})_3$  procedure does recover some large viruses, and the protamine sulfate procedure does recover some small viruses. Thus, the results from the  $\text{Al}(\text{OH})_3$  procedure and those from the protamine sulfate procedure overlap somewhat and are not additive when separate effluent samples were used for each. Unless otherwise indicated in the table footnote, however, the two procedures were applied in tandem on the same sample, and the total number of viruses recovered reflect the minimum present in the sample. In any event, neither procedure is quantitative. The total amount of viruses in an effluent must exceed by some considerable amount the quantity of viruses detected.

Recovery of viruses from river water. Fifty or 100 gallons of river water were filtered through a 293 mm Tween-80-treated Millipore AP 20 prefilter and then through two 143 mm Tween-80-treated AP 20 prefilters between which were sandwiched 1.8 gm of washed Monsanto PE 60 polyelectrolyte (4). The filter pads were supported in Millipore filter holders of appropriate size connected in tandem. The 293 mm filter clogged frequently with silt and was replaced as necessary. The silt was collected with a spatula and the virus eluted from it with 3% beef extract by a method designed in this laboratory (6). Viruses were eluted from the polyelectrolyte by circulating 60 ml of 0.5% pancreatin through the sandwich three times, and then circulating 60 ml of pH 9 borate buffer containing 10% fetal calf serum through the sandwich three times. The borate buffer consisted of 0.05 M  $\text{H}_3\text{BO}_3$ ,

0.05 M KCl, and sufficient NaOH to bring the pH to 9. Each eluate was collected separately, filtered through an MF 0.45- $\mu$  membrane filter to remove bacteria and fungi, and inoculated onto cell cultures, 1 ml per culture for assay of viruses.

Cell cultures. All viruses were isolated by the plaque technic in primary cell cultures prepared from rhesus monkey kidney cells.

Identification of viruses. Viruses are being identified under contract with Dr. S. S. Kalter, Southwest Foundation for Research and Education, San Antonio, Texas.

#### RESULTS AND DISCUSSION

Our first important effort to demonstrate virus pollution of a major stream was undertaken in the late summer of 1969 along the Missouri River. Repeated efforts to demonstrate, with standard pollution indicators [such as depressed dissolved oxygen levels (DO) and 5-day 20 C biochemical oxygen demands (BOD)] deleterious alteration of the stream by communities that discharged primary effluents into it had been essentially unsuccessful. The likelihood of recovering viruses from a stream when other indicators of pollution could not be demonstrated seemed remote: Viruses usually do not reach levels much beyond several thousand plaque-forming units (PFU)\* per gallon of sewage, they do not multiply in the effluent or in the stream, and they slowly die off as time progresses. Moreover, once diluted in the stream they become difficult to detect because good quantitative concentration methods have not yet been developed. There did exist, however, the advantage that demonstration of even one virus particle of

\* Plaque-forming units are infectious units.

human origin is bonafide evidence of dangerous pollution, whereas small changes in pollution indicator values below outfalls are difficult to interpret.

Thus, our initial efforts were directed at detecting viruses in effluents discharged into the river. Six times during the months of September and October 1969, samples were taken from sewage treatment plants along stretches of the waterway. Most samples were primary effluent, but some raw sewage and river water samples were also taken. Viruses, often several hundred per gallon, were consistently recovered from municipal treatment plant primary effluents, and from raw sewage (Tables 2-7). Stock yard effluents also yielded hundreds of viruses when tested in calf kidney cells, indicative that animal viruses in large numbers are continuously discharged into the waterway. In itself, all of this was bonafide evidence that the Missouri River was being polluted with infectious agents.

To determine how far downstream these infectious agents constituted a hazard presented a more complex problem, because dilution of the viruses in the stream necessitates a concentration of small numbers of viruses from very large volumes of water. No established methods were available for such studies. As an alternative, viruses of the types present in sewage effluents (enteroviruses and reoviruses) were seeded into Missouri River water and into the effluents as well, and 24-hour viral survivals (long enough to reach major downstream water supplies) were determined. Table 8 shows that large numbers of viruses survived in the Missouri River water, in the sewage, and in the effluents

after 24 hours. The reovirus, reportedly capable of producing cancers in certain animals when inoculated in very small amounts (5), appeared to increase in numbers in the river water, perhaps the result of clumps breaking up. It was clear, in any event, that viruses ejected into waterways with domestic sewage could reach water intakes many miles downstream.

The detection of viruses at water intakes downstream of a pollution source can demonstrate that the hazard perpetrated in the area of an outfall by the discharge of viruses into that area has extended itself to the downstream community. Good quantitative technics for detecting small amounts of viruses in large volumes of water were not available, but a technic under development, capable of detecting a portion of the viruses present, gave some promise of sufficient sensitivity. This was the polyelectrolyte method. In this method (see Materials and Methods), large volumes of water are filtered through a Monsanto compound designated PE 60 which adsorbs some viruses. Adsorbed viruses subsequently can be eluted and quantified. Thus, an effort was made to recover viruses from large volumes of river water. An attempt was made also to recover viruses from the silt that collected on fiberglass prefilters used to remove suspended material before the water passed through the polyelectrolyte. A number of attempts were made to detect viruses upstream and downstream from outfalls and at some water intakes. These studies are summarized in Table 9.

A 50-gallon water sample taken at Missouri City yielded five viruses, and a sample of similar size taken at Bellevue yielded four

viruses. Six more viruses were recovered from the silt in the water sample. At Sioux City, four viruses were recovered from a 50-gallon water sample and its silt five miles below the sewage outfalls, but none were recovered from samples taken above the outfalls. Forty-eight viruses per liter of sample were recovered from Sioux City primary effluent sampled on the same day.

A mid-winter study at St. Joseph yielded interesting results. One virus was recovered from 50 gallons of water taken at Palermo landing, about 10 miles below St. Joseph's sewage treatment outfalls, but 19 viruses were recovered from a sample of similar size taken at the water intake above the outfalls. This is equal to 360,000 viruses per million gallons of water. Recovery of viruses from water intakes during the winter, especially, when transmission of enteric viruses is at a relatively low point, underscores the hazard perpetrated upon downstream communities by upstream communities that discharge viruses in their effluents. St. Joseph primary effluents also yielded considerable quantities of viruses (Table 9). This study was repeated in the spring at which time viruses were again recovered from the water intake, and at Palermo landing also.

All viruses identified thus far are polioviruses and echoviruses, are of human origin, and are capable of infecting humans who consume them.

Since trucking multiple 50-gallon samples to Cincinnati, especially during the winter months, was difficult, a comparative study was set up at the time of the spring sampling at St. Joseph to determine whether

filtrations through the polyelectrolyte could be done in the field and virus elution and isolation subsequent to return of the filters to the Cincinnati laboratory. The results of this study show that the same amount of viruses were recovered when filtrations were done in the field as when they were done in the laboratory on trucked-in samples (Table 10). Subsequent studies in our laboratory showed that storage of the filters at 4 C for several days before and after water was filtered through them did not reduce virus recoveries (Table 11), and thus added support to the feasibility of a field filtration technic. The results in Table 11 also demonstrate the inefficiency of the technic as a quantitative method for virus recovery.

Although technical problems occurred in the field that made it impossible to filter much more than half of the 50 gallons that were filtered in the laboratory, these problems were minor and should be easy to resolve.

#### SUMMARY

A single viable virus excreted by a human is capable of infecting other humans who consume it, and thus constitutes a hazard to health and well-being. Thus, each virus is a dangerous pollutant. Viruses have been detected along the Missouri River in effluents, midstream, and at water intakes, demonstrating a clearcut hazard perpetrated upon downstream communities by those upstream. The methods used to demonstrate the virus pollution were capable of detecting only a portion of the viruses present. Thus, many more viruses were present than we were able to detect. There is clearly an urgent need for developing better methodology for detecting small numbers of viruses in large volumes of water.

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Table 1. Minimal Infective Dose of Viruses for Man

| Virus              | Dose*               | Route of inoculation      | No. infected<br>No. inoculated | % infected |
|--------------------|---------------------|---------------------------|--------------------------------|------------|
| Poliovirus 1 (SM)  | 2 PFU               | Oral<br>(gelatin capsule) | 2/3                            | 67         |
| Poliovirus 3 (Fox) | 1 TCD <sub>50</sub> | Gavage                    | 3/10                           | 30         |
| Measles            | 1 TCD <sub>50</sub> | Intranasal                | 8/35                           | 24         |

\* Given in plaque-forming units (PFU) or that amount of virus that will infect 50% of the tissue cultures inoculated (TCD<sub>50</sub>).

Table 2. Recovery of Viruses from Missouri River Effluents  
(Samples of 9/19/69)

| Sample | Site of sampling<br>Place                                        | River miles | Type of<br>effluent | Laboratory<br>recovery<br>method | Virus<br>recovered<br>(PFU/liter) |
|--------|------------------------------------------------------------------|-------------|---------------------|----------------------------------|-----------------------------------|
| A-25.5 | Atchison sewage<br>treatment plant                               | 421.0       | Primary             | Al(OH) <sub>3</sub>              | 9.5                               |
| I-24.5 | Leavenworth sewage<br>treatment plant                            | 395.6       | Primary             | Al(OH) <sub>3</sub>              |                                   |
| SJ-15  | St. Joseph sewage<br>treatment plant                             | 446.4       | Primary             | Al(OH) <sub>3</sub>              | 8                                 |
| SJ-18  | South St. Joseph<br>industrial sewer<br>district (stock<br>yard) | 445.6       | Primary             | Al(OH) <sub>3</sub> <sup>*</sup> | 2.5                               |
|        |                                                                  |             |                     | Protamine sulfate <sup>*</sup>   | 10                                |
|        |                                                                  |             |                     | Al(OH) <sub>3</sub>              | 0                                 |
|        |                                                                  |             |                     | Protamine sulfate                | 4                                 |

\* Tested in calf kidney cells.

Table 3. Recovery of Viruses from Missouri River Effluents  
(Samples of 9/24/69)

| Sample | Site of sampling<br>Place                                        | River miles | Type of<br>effluent | Laboratory<br>recovery<br>method                                                        | Virus<br>recovered<br>(PFU/liter) |
|--------|------------------------------------------------------------------|-------------|---------------------|-----------------------------------------------------------------------------------------|-----------------------------------|
| A-25.5 | Atchison sewage<br>treatment plant                               | 421.0       | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate                                                | 128.5<br>274                      |
| L-24.5 | Leavenworth sewage<br>treatment plant                            | 395.6       | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate                                                | 50<br>9                           |
| SJ-15  | St. Joseph sewage<br>treatment plant                             | 446.4       | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate                                                | 53<br>20.5                        |
| SJ-18  | South St. Joseph<br>industrial sewer<br>district (stock<br>yard) | 445.6       | Primary             | Al(OH) <sub>3</sub> *<br>Protamine sulfate*<br>Al(OH) <sub>3</sub><br>Protamine sulfate | 45.5<br>58<br>85<br>15            |

\* Tested in calf kidney cells.

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Table 4. Recovery of Viruses from Missouri River Effluents  
(Samples of 9/26/69)

| Sample | Site of sampling<br>Place                                          | River miles | Type of<br>effluent | Laboratory<br>recovery<br>method <sup>+</sup> | Virus<br>recovered<br>(PFU/liter) |
|--------|--------------------------------------------------------------------|-------------|---------------------|-----------------------------------------------|-----------------------------------|
| M-19   | Big Blue River<br>sewage treatment<br>plant.                       |             | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate      | 96<br>18                          |
| M-104  | Kansas City, Mo.<br>westside sewage<br>treatment plant             | 367.19      | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate      | 71<br>0.5                         |
| M-106  | Rock Creek below<br>North Kansas City<br>sewage treatment<br>plant | 362.7       | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate      | 2.5<br>0.5                        |
| M-108  | Rock Creek at<br>Independence, Mo.<br>raw sewage by-pass<br>line   | 356.9       | Raw<br>sewage       | Al(OH) <sub>3</sub><br>Protamine sulfate      | 85<br>9                           |
| M-102  | Kaw Valley District<br>outfall                                     | 367.6L      | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate      | 61<br>2                           |
| M-103  | Kansas City, Kansas<br>sewage treatment<br>plant                   | 367.2L      | Primary             | Al(OH) <sub>3</sub><br>Protamine sulfate      | 73<br>2.5                         |

<sup>+</sup> Separate samples used for tests with Al(OH)<sub>3</sub> and protamine sulfate.

Table 5. Recovery of Viruses from Missouri River Effluents  
(Samples of 10/1/69)

| Sample | Site of sampling<br>Place River miles                           | Type of effluent | Laboratory recovery method               | Virus recovered (PFU/liter) |
|--------|-----------------------------------------------------------------|------------------|------------------------------------------|-----------------------------|
| M-19   | Big Blue River sewage treatment plant                           | Primary          | Al(OH) <sub>3</sub><br>Protamine sulfate | 109<br>0                    |
| M-102  | Kaw Valley District outfall 367.6L                              | Primary          | Al(OH) <sub>3</sub><br>Protamine sulfate | 0<br>1                      |
| M-103  | Kansas City, Kansas sewage treatment plant 367.2L               | Primary          | Al(OH) <sub>3</sub><br>Protamine sulfate | 146<br>56                   |
| M-104  | Kansas City, Mo. westside sewage treatment plant 367.19         | Primary          | Al(OH) <sub>3</sub><br>Protamine sulfate | 92<br>6                     |
| M-106  | Rock Creek below north Kansas City sewage treatment plant 362.7 | Primary          | Al(OH) <sub>3</sub><br>Protamine sulfate | 1.5<br>0                    |
| M-108  | Rock Creek at Independence, Mo. raw sewage by-pass line 356.9   | Raw sewage       | Al(OH) <sub>3</sub><br>Protamine sulfate | 16<br>0.5                   |

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Table 6. Recovery of Viruses from Missouri River Effluents  
(Samples of 10/16/69)

| Sample | Site of sampling<br>Place                                                          | River miles | Type of<br>effluent             | Laboratory<br>recovery<br>method*        | Virus<br>recovered<br>(PFU/liter) |
|--------|------------------------------------------------------------------------------------|-------------|---------------------------------|------------------------------------------|-----------------------------------|
| OM-40A | Omaha-Missouri<br>River sewage<br>treatment plant<br>from Monroe Street<br>by-pass | 611.5       | Raw sewage                      | Al(OH) <sub>3</sub><br>Protamine sulfate | 19<br>222                         |
| M-211  | Pacific Fruit<br>Express outfall                                                   | 611.5R      | Train car wash<br>raw discharge | Al(OH) <sub>3</sub><br>Protamine sulfate | 0<br>0                            |
| M-212  | Quaker Oats Co.                                                                    | 615.2L      | Raw process<br>waste<br>outfall | Al(OH) <sub>3</sub><br>Protamine sulfate | 0<br>0                            |

\* Separate samples used for tests with Al(OH)<sub>3</sub> and protamine sulfate.

Table 7. Recovery of Viruses from Missouri River Effluents  
(Samples of 10/24/69)

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| Sample | Site of sampling<br>Place River miles                | Type of effluent             | Laboratory recovery method                                         | Virus recovered (PFU/liter) |
|--------|------------------------------------------------------|------------------------------|--------------------------------------------------------------------|-----------------------------|
| M-38   | Bellevue, 0.1 miles above State Highway 370 bridge   | River water                  | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 0                           |
| M-203  | Omaha-Papillion Creek sewage treatment plant         | 2/3 secondary<br>1/3 primary | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 0                           |
| M-211  | Pacific Fruit Express                                | Train car wash raw discharge | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 41                          |
| M-212  | Quaker Oats Co.                                      | Raw process waste outfall    | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 1.5                         |
| OM-40A | Omaha-Missouri River sewage treatment plant          | Primary                      | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 0                           |
| TC-210 | Twin Cities Plaza raw sewage discharge               | Raw sewage                   | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 20                          |
| CB-40B | Council Bluffs sewage treatment plant                | Primary                      | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 26                          |
| OM-208 | Monroe Street bypass of raw wastes to Missouri River | Raw packing waste            | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 5                           |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 7                           |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 135                         |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 286                         |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 95                          |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>+</sup><br>Protamine sulfate <sup>+</sup> | 201                         |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>+</sup><br>Protamine sulfate <sup>+</sup> | 0                           |
|        |                                                      |                              | Al(OH) <sub>3</sub> <sup>+</sup><br>Protamine sulfate <sup>+</sup> | 0.5                         |

\* Separate samples used for tests with Al(OH)<sub>3</sub> and protamine sulfate.

+ Tested in calf kidney cells.

Table 7. Recovery of Viruses from Missouri River Effluents  
(Samples of 10/24/69)

| Sample | Site of sampling<br>Place River miles                       | Type of effluent             | Laboratory recovery method                                         | Virus recovered (PFU/liter) |
|--------|-------------------------------------------------------------|------------------------------|--------------------------------------------------------------------|-----------------------------|
| M-38   | Bellevue, 0.1 miles above State Highway 370 bridge          | River water                  | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate              | 0                           |
| M-203  | Omaha-Papillion Creek sewage treatment plant                | 2/3 secondary<br>1/3 primary | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 0<br>41                     |
| M-211  | Pacific Fruit Express 611.5R                                | Train car wash raw discharge | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 1.5<br>0                    |
| M-212  | Quaker Oats Co. 615.2L                                      | Raw process waste outfall    | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 0<br>0                      |
| OM-40A | Omaha-Missouri River sewage treatment plant 611.5L          | Primary                      | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 20<br>26                    |
| TC-210 | Twin Cities Plaza raw sewage discharge 613.6R               | Raw sewage                   | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 5<br>7                      |
| CB-40B | Council Bluffs sewage treatment plant 614.0R                | Primary                      | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 135<br>286                  |
| OM-208 | Monroe Street bypass of raw wastes to Missouri River 611.4L | Raw packing waste            | Al(OH) <sub>3</sub> <sup>*</sup><br>Protamine sulfate <sup>*</sup> | 95<br>201                   |
|        |                                                             |                              | Al(OH) <sub>3</sub> <sup>+</sup><br>Protamine sulfate <sup>+</sup> | 0<br>0.5                    |

\* Separate samples used for tests with Al(OH)<sub>3</sub> and protamine sulfate.

+ Tested in calf kidney cells.

| Samples<br>collected | Site of sampling<br>Place/River miles               | Type of<br>sample            | Poliovirus 1          |                         | Echovirus 7        |                         | Reovirus 1         |                         |
|----------------------|-----------------------------------------------------|------------------------------|-----------------------|-------------------------|--------------------|-------------------------|--------------------|-------------------------|
|                      |                                                     |                              | Hours<br>0            | Hours<br>24             | Hours<br>0         | Hours<br>24             | Hours<br>0         | Hours<br>24             |
| 59                   | River<br>water<br>354.4                             | Water                        | $9.6 \times 10^{4**}$ | $7.1 \times 10^4(74)**$ | $9.5 \times 10^4$  | $4.7 \times 10^4(50)$   | $1.6 \times 10^3$  | $1.07 \times 10^4(167)$ |
|                      | Big Blue<br>River sewage<br>treatment plant         | Primary<br>effluent          | $11.3 \times 10^4$    | $6.0 \times 10^4(53)$   | $8.8 \times 10^4$  | $13.2 \times 10^4(150)$ | $2.4 \times 10^3$  | $2.06 \times 10^3(86)$  |
| 69                   | River<br>water<br>601.3                             | Water                        | $1.1 \times 10^5$     | $6.8 \times 10^4(61)$   | $8.7 \times 10^4$  | $8.0 \times 10^4(92)$   | $1.21 \times 10^3$ | $1.65 \times 10^3(135)$ |
|                      | Papillion<br>Creek<br>596.6                         | 2/3 secondary<br>1/3 primary | $1.12 \times 10^5$    | $4.4 \times 10^4(40)$   | $7.6 \times 10^4$  | $1.04 \times 10^5(136)$ | $9.4 \times 10^2$  | $2.23 \times 10^3(238)$ |
| 59                   | River<br>water<br>440.3                             | Water                        | $1.47 \times 10^5$    | $3.2 \times 10^4(22)$   | $1.44 \times 10^5$ | $7.8 \times 10^4(54)$   | $3.2 \times 10^3$  | $2.15 \times 10^4(672)$ |
|                      | S. St.<br>Joseph<br>ind.<br>sewer<br>dist.<br>445.6 | Primary<br>effluent          | $1.34 \times 10^5$    | $1.22 \times 10^5(91)$  | $1.2 \times 10^5$  | $9.5 \times 10^4(79)$   | $5.2 \times 10^3$  | $3.39 \times 10^3(65)$  |
| 69                   | River<br>water<br>M-48<br>718.3                     | Water                        | $3.12 \times 10^5$    | $5.8 \times 10^4(19)$   | $1.6 \times 10^5$  | $5.6 \times 10^4(35)$   | $1.25 \times 10^4$ | $5.1 \times 10^4(408)$  |
|                      | Sioux<br>City<br>SC-49<br>729.0R                    | Primary<br>effluent          | $1.89 \times 10^5$    | $8.4 \times 10^4(44)$   | $1.34 \times 10^5$ | $6.8 \times 10^4(51)$   | $1.4 \times 10^4$  | $1.52 \times 10^4(109)$ |

\* Samples were stored at room temperature (23-26 C).

\*\* PFU per ml.

\*\* Percent surviving given in parenthesis.

Table 9. Recovery of Viruses from Missouri River Water and Silt

| Date of sampling | Site of sampling<br>Place                                  | River miles | Type of sample   | Size of sample  | Lab recovery procedure | Virus recovered (PFU/sample) | Virus types recovered*       |
|------------------|------------------------------------------------------------|-------------|------------------|-----------------|------------------------|------------------------------|------------------------------|
| 10/8/69          | Missouri City Power Plant Dock                             | 354.4       | Water            | 50 gal          | Polyelectrolyte        | 5                            | E8, E8, EL, EL, E7           |
|                  |                                                            |             | Silt             | from 8.33 gal** | Beef extract           | 0                            |                              |
| 10/30/69         | Near Omaha Bellevue Power Plant                            | 601.3       | Water            | 50 gal          | Polyelectrolyte        | 4                            | P2, E7, P2,                  |
|                  |                                                            |             | Silt             | from 50 gal**   | Beef extract           | 6                            | EL, P1, E7, P3               |
| 12/11/69         | Thacker Marina in Sioux City (above all sewage outfalls)   | 732.7L      | Water            | 50 gal          | Polyelectrolyte        | 0                            |                              |
|                  | Sioux City                                                 |             | Silt             | from 50 gal**   | Beef extract           | 0                            |                              |
|                  |                                                            |             | Primary effluent | 2 liters        | Al(OH) <sub>3</sub>    | 82                           | Not yet typed                |
|                  |                                                            |             |                  |                 | Protamine sulfate      | 14                           | Not yet typed                |
|                  | Sioux City (5 miles below all sewage outfalls)             | 717.0       | Water            | 50 gal          | Polyelectrolyte        | 1                            | Not yet typed                |
|                  |                                                            |             | Silt             | from 50 gal**   | Beef extract           | 3                            | P3, P3                       |
| 1/22/70          | St. Joseph Water Works intake line (above sewage outfalls) | 452.3       | Water            | 50 gal          | Polyelectrolyte        | 19                           | P3, P2, P2, P2, E7, E33, E33 |
|                  | St. Joseph Municipal                                       |             | Silt             | from 50 gal**   | Beef extract           | 0                            |                              |
|                  |                                                            |             | Primary effluent | 2 liters        | Al(OH) <sub>3</sub>    | 88                           | Not yet typed                |
|                  |                                                            |             |                  |                 | Protamine sulfate      | 5                            | Not yet typed                |
|                  | St. Joseph Municipal (meat packing plant)                  |             | Primary effluent | 2 liters        | Al(OH) <sub>3</sub>    | 134                          | Not yet typed                |
|                  |                                                            |             |                  | -               | Protamine sulfate      | 0                            |                              |
|                  | Palermo Landing                                            | 440.3       | Water            | 50 gal          | Polyelectrolyte        | 1                            | E33                          |
|                  |                                                            |             | Silt             | from 50 gal**   | Beef extract           | 0                            |                              |

4/23/70

|                                                                   |       |                  |               |                     |     |               |
|-------------------------------------------------------------------|-------|------------------|---------------|---------------------|-----|---------------|
| St. Joseph Water Works intake (above sewage outfalls)             | 452.3 | Water            | 50 gal        | Polyelectrolyte     | 3   | Not yet typed |
|                                                                   |       | Silt             | from 50 gal** | Beef extract        | 0   |               |
| St. Joseph Municipal sewage treatment plant                       |       | Primary effluent | 2 liters      | Al(OH) <sub>3</sub> | 222 | Not yet typed |
|                                                                   |       |                  |               | Protamine sulfate   | 66  | Not yet typed |
| St. Joseph Industrial sewage treatment plant (meat packing plant) |       | Primary effluent | 2 liters      | Al(OH) <sub>3</sub> | 10  | Not yet typed |
|                                                                   |       |                  |               | Protamine sulfate   | 0   |               |
| Palermo Landing                                                   | 440.3 | Water            | 50 gal        | Polyelectrolyte     | 3   | Not yet typed |
|                                                                   |       |                  | from 50 gal** | Beef extract        | 0   |               |

\* Not all viruses recovered have been typed as yet.

\*\* Silt taken from prefilters used in filtration of 50-gallon samples. Sometimes silt from volumes less than the 50 gallons filtered were used.

21  
Table 10. Comparison of Field Filtration and Laboratory Filtration of Water Samples on Efficiency of Recovery of Viruses from Missouri River Water at St. Joseph

| Site of sampling<br>Place River miles | Type of<br>sample | Size of<br>sample    | Site of<br>filtration | Virus<br>recovered<br>(PFU/sample) | Virus<br>types **<br>recovered |
|---------------------------------------|-------------------|----------------------|-----------------------|------------------------------------|--------------------------------|
| Watervorks 452.3<br>Intake            | Water             | 50 gal               | Laboratory            | 3                                  |                                |
|                                       |                   | 3l gal               | Field                 | 1                                  | E1                             |
|                                       | Silt              | Silt from<br>50 gal* | Laboratory            | 0                                  |                                |
| Palermo<br>Landing 440.3              |                   | Silt from<br>3l gal* | Field                 | 1                                  | E7                             |
|                                       | Water             | 50 gal               | Laboratory            | 3                                  |                                |
|                                       |                   | 2l gal               | Field                 | 1                                  | E7                             |
|                                       |                   | Silt from<br>50 gal* | Laboratory            | 0                                  |                                |
|                                       | Silt              | Silt from<br>2l gal* | Field                 | 2                                  | E7                             |

\* Silt taken from prefilters used in filtration of corresponding water samples.

\*\* Not all viruses recovered have been typed as yet.

82.  
Table 11. Recovery of Viruses from Freshly Prepared and Stored Polyelectrolyte PE 60

| Virus        | Control | Freshly prepared PE 60 | Stored PE 60 | Storage (days at 4 C) |            |
|--------------|---------|------------------------|--------------|-----------------------|------------|
|              |         |                        |              | Before test           | After test |
| Poliovirus 1 | 75*     | 38* (51%)              | 45* (53%)    | 2                     | 3          |
| Echovirus 7  | 79      | 24 (30%)               | 20 (25%)     | 2                     | 5          |
| Reovirus 1   | 105     | 33 (31%)               | 9 (8%)       | 2                     | 5          |
| Reovirus 1   | 84      | 14 (17%)               | 12 (14%)     | 2                     | 2.5        |

\* Plaque-forming units.